AN HISTOLOGICAL STUDY OF REGENERATIVE PHENOMENA IN PLANTS[1]

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Introduction

Nemec ('05), Pfeffer ('05), and Prantl ('74) employ the word regeneration in its narrowest sense when they consider that it is the formation of new parts exactly alike in number and position to the organs injured or removed. Since new structures generally originate only from actively growing tissues, regeneration is practically limited to the embryonic tissues of the root and shoot. For example, if an old root tip is removed, the new root tip is regenerated. Roots forming on the stem, however, are not regenerated roots but simply adventitious roots. An example of this type is the root developed at the nodes of *Tradescantia* when that plant is placed in a glass of water. The extreme opposite of this view has been held by Vöchting ('78), Goebel ('02), Morgan ('01), and Klebs ('03) who consider the development of dormant buds present on the part before injury to be a regeneration phenomenon. They, therefore, include in their definition of regeneration a phase of normal vegetative growth which might be termed merely the stimulation of bud development, the production of new roots or new buds, etc., in any position in which these organs do not normally occur. A more moderate view, however, is assumed by Miss Kupfer ('07) who says that regeneration "ought to be limited to organs formed 'de novo' at a place or under conditions not normally so [formed]." Therefore, she excludes latent root and shoot development which occur, for example, when a willow twig is placed in the ground, and as would be included in the definition by Goebel ('02), Vöchting ('78), and Morgan ('01).

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of master of arts in the Henry Shaw School of Botany of Washington University.

SUMMARY OF LITERATURE AS REGARDS TISSUES INVOLVED IN REGENERATION

The work on regeneration has dealt primarily with the organographic aspect and also with the theories as to the cause of the releasing stimuli in the phenomena. Some studies have laid stress upon the external influences to which the plant has been subjected and the relation of these to the production of regenerative processes; likewise, the tendencies inherent in the plant itself have been duly considered. Therefore, a relatively small amount of literature deals with the tissues concerned in regeneration. Prantl ('74) found that in the case of corn and *Vicia Faba* regeneration was effected through the intermediary of a common callus which represented the passage of the different systems into one another. Moreover, he found no differentiation in the callus tissue of specific groups representing the diverse tissues of the root. In 1900, and later in 1902 and 1903, Goebel conducted elaborate experiments on *Taraxacum*, *Bryophyllum crenatum*, and other plants. His conclusions were concerned chiefly with the physiological characters of regeneration, its causes and effects. Simon, in 1904 and later in 1908, after investigating the regeneration of root tips and shoots, stated that all tissues may indirectly form a callus and then regeneration may take place or, directly, each tissue may form a callus. McCallum's work in 1905 on species of *Phaseolus*, *Salix*, *Helianthus*, *Taraxacum*, and other plants was of a physiological nature, dealing with the disturbances in nutrition, disturbance in water content, wound stimuli, and correlation. Nemec ('05) made an elaborate study of the regeneration of roots and found regenerated parts developed from either a callus formation or directly from the division of the cells at the base of the dermatogen. Miss Kupfer ('07) found that regeneration of fleshy roots developed from the cambium and callus, as in the horseradish, sweet-potato, and parsnip. She makes no reference to the sectioning or preparation of slides of her material. Therefore, from these studies, organ regeneration is dependent upon the cambial and callus tissues.

Experimental Work

Object.—The object of this work is to secure regeneration, to find the earliest stages of the divisions of those cells giving rise to the regenerated parts, and to trace the development of those tissues and the relation of those regenerated parts to the tissues of the original plant by means of histological study.

Methods and materials.—The materials used were flax seedlings, pieces of sweet-potato, horseradish, parsnips, tobacco stems and buds, and *Bryophyllum* leaves. The work was carried on with sterile sand cultures, water cultures, and potted cultures. Care was taken to use sterile cultures that the materials might be kept a longer period of time than in previous studies free from molds, bacteria, etc. Sterile cultures necessitated the use of nutrient solutions. The nutrient solutions used were Shive's solution (A) and Duggar's solution (B).

The following cultures were set up in test-tubes, Ehrlenmeyer flasks, and quart jars, all being plugged with cotton and then sterilized in the autoclave at 15 pounds pressure for 20 minutes. Tumblers were inserted over the jars that no dust might sift through the cotton. The test-tubes were kept in a moist chamber to keep them free from dust.

FIRST SET OF EXPERIMENTS

Experiment 1.—Ordinary tap-water was used for the water cultures. A 3-inch piece of a horseradish root was cut at the top and bottom and suspended in a tumbler of water by means of a wire. In about a week roots and shoots developed. Upon examination they were found to have developed from the cambium.

Experiment 2.—A piece of a horseradish root was peeled so as to leave nothing but the pith. This was suspended in a tumbler of water which was continually refilled. After 9 days a few regenerated shoots developed, but upon examination they were found to have originated from a few adhering cambium cells.

Experiment 3.—A whole sweet-potato was suspended by a wire in a quart-jar of water. Within 10 days many shoots developed normally from the cambium.

Experiment 4.—The pith of a sweet-potato, similarly suspended in a jar of water, exhibited no regeneration even after 2 months. Wherever regeneration occurred in any of these cultures, the regenerated parts were cut out with as little of the root proper as was necessary and put in killing solutions as later described in preparation for histological study. Even those portions which gave only a very slight indication of the possibility of regeneration were used.

SECOND SET OF EXPERIMENTS

About 10 cc. of sand were added to each of 4 test-tubes. To 2 was added solution A and to the other two, solution B. These were sterilized and, according to the preparation of sterile cultures, portions, about 1 cm. square, of tobacco leaves and buds were added. No regeneration occurred even after one month. Similar cultures, using pieces of sweet-potato instead of tobacco, were set up, but these, too, exhibited no regeneration. These same experiments were repeated a few days later but with no results. Either the sections were too small or the nutrient solutions were lacking in some way or some other factor prevented the regeneration which, according to Miss Kupfer, developed under non-sterile conditions. A few weeks later similar experiments were set up, using tobacco leaves and buds and pieces of sweet-potato. In this case, however, a few cc. of 1 per cent cane sugar and 1 per cent peptone solutions were added to the nutrient solutions. No regeneration occurred, and this brought up the possibility of using larger pieces. Accordingly, pieces of parsnip, horseradish, and sweet-potato, about 1 cm. in thickness, were placed in sterile sand cultures in ordinary quart-jars. These jars were plugged with cotton and over them tumblers were inserted. To these solutions were also added a few cc. of 1 per cent peptone and 1 per cent cane-sugar solutions. The radish and potato roots were soaked in Javelle water for 2 hours before sectioning, to render the outer surfaces sterile. Within about a week all pieces developed regenerated roots or shoots. The early stages of these regenerated parts were removed in as small portions as possible and fixed for microscopic study.

THIRD SET OF EXPERIMENTS

(1) An entire horseradish root was covered with soil.

(2) A root of the horseradish was peeled down to the pith and covered with soil.

(3) Horseradish roots were cut in half longitudinally and horizontally and were placed in the soil.

(4) Similar pieces of the sweet-potato were used.

(5) The peelings of the radish and potato were planted. After one week all pieces, except the peels, exhibited regenerated roots or shoots. As previously described, these parts were removed, fixed, and preserved for study.

(6) Four detached *Bryophyllum* leaves were placed in a moist chamber on wet sand with the bases of the petioles covered. Every notch exhibited regenerated shoots and roots. The earliest stages of these were cut off and killed.

Perhaps the most interesting regeneration was developed by the flax seedlings. The flax seeds were repeatedly planted in porous saucers containing a mixture of sand and soil, and kept in moist chambers. After the unfolding of the cotyledons, these seedlings were decapitated about 1–2 cm. beneath the cotyledons. In some instances where the roots were above the soil, these too were cut off. After 6 to 10 days, each stem exhibited tiny swellings, as many as 8 appearing on one stem. Soon these swellings developed into shoots. When the root was cut off, these swellings appeared at the base of the stem and soon developed into new roots. As many as 4 regenerated roots developed on one plant. The stems that exhibited swellings and regenerated roots and shoots were cut into small sections so that each section had one swelling or regenerated root or shoot. These sections were then fixed as described below.

After washing, the materials were dehydrated, infiltrated, sectioned, and mounted in balsam according to the method given in Chamberlain's 'Methods in Plant Histology.' Some of the sections were stained in Delafield's haematoxylin and some in saffranin.

As stated before, after some regeneration was evident with the hand lenses, those sections and those giving slight indication of the possibility of regeneration as described before were killed with the following solutions:

Materials	Solution	Time	Wash
Flax	Chromo-acetic (0.5 gm. chromic acid 1.0 cc. glacial acetic 100 cc. H_2O)	12 hr.	12 hr. running H_2O
Flax Sweet-potato Horseradish Parsnip	Corrosive sublimate + acetic acid (1.2 g. sublimate 3.0 cc. glacial acetic 100 cc. alcohol of, (a) 5% or (b) 15% or (c) 95%)	3–4 hr.	12 hr., (a) 5% alcohol or (b) 15% alcohol or (c) 95% alcohol

The plates, with the exception of pl. 15, illustrate the histological study. Plate 15 shows: (1) a normal flax seedling, one with decapitated roots, and one with the cut-off cotyledons, with the regenerated shoots and roots as a result of decapitation; (2) the notches of *Bryophyllum* leaf were the places at which occurred regeneration; (3) a sweet-potato developed roots from the cambium on the cut surface and normally through the cortex and epidermis; (4) the horseradish exhibited a similar condition to the potato.

Plate 16 shows: (1) a cross-section of a flax stem with the central cylinder of phloem and xylem cells but no well-defined cambium tissue, the large irregular cortex cells, and the small, more regular epidermal cells; (2) *b* represents the earliest stages in the division of the epidermal cells to form a swelling; *c*, *d*, and *e* show the continued divisions to form a larger swelling, which finally would result in the formation of a true bud which would then seek direct connection with the central cylinder of the stem. Thus, the origin of the regenerated shoot in the flax is from the division of the epidermal cells followed by the division of the cells thus formed. There is the possibility that if there were a definite cambium that regeneration would originate from those cells, as in the radish, parsnip, etc., when the cambium is present and, undoubtedly give rise to the regenerated parts.

Plate 17 shows the regenerated root of the horseradish and the regenerated bud of the sweet-potato. Because the sections of both the potato and radish exhibited similar regenerative phenomena, only one case from each has been drawn: *a* shows a well-

differentiated group of meristem cells within the section of the radish. The origin of these cells is not shown, but it is plain that it cannot be from the epidermis or cortical layer, since these layers completely enclose the regenerating tissues; *b* shows a similar group of regenerating cells with a direct connection to the cambium of the root. Since, then, this group shows this connection, it seems that its origin must be from those cells or the cambium. *c* shows the bud enclosed in a similar manner. Other slides exhibited buds likewise enclosed by the epidermis and cortex and with a direct connection to the cambium. Hence, shoots, too, must originate from the division of cambium cells.

Sections through the vegetative points of the *Bryophyllum* leaf are shown in pl. 18: *a* represents a section through a normal portion showing a vein; *b* is an enlarged section of the vein with the division of the small phloem cells; *c* shows that division carried still farther until it disfigures the leaf by a tiny swelling, which results, as in *d*, in the formation of a regenerated root and shoot. Thus the root and shoot arise from the division of the small phloem cells of the vein near the vegetative points or notches of the leaf.

General Results

After about 5 or 10 days, there appeared roots and shoots on the horseradish, parsnip, and sweet-potato in the sterile sand cultures. Buds appeared after 10 days on the radish and potato cuttings which were first washed in Javelle water. Similar results were noted in the water cultures and sand cultures. After the cotyledons of the flax seedlings had been removed, there appeared tiny buds of shoots on the stem, and when the roots had been removed new roots were regenerated (pl. 15). Every notch of the *Bryophyllum* leaves regenerated new roots and shoots. The tobacco cuttings failed to regenerate and decayed. It was difficult to render them sterile on account of the many hairs on the surface. Freehand sections were made of the parsnip, horseradish, and sweet potato, and stained with iodine. It was plainly evident that the regenerated part was connected with the cambium, the cells of which took on the characteristic color of

protoplasm stained with iodine while the surrounding cells were filled with purple-stained starch grains (pl. 15). From the prepared sections, it can be seen that, according to pl. 17, the regenerated parts of the potato and radish came from tissues other than the epidermis or cortex. The one drawing on this plate shows the definite connection with the cambium. The parsnip slides exhibited an exactly similar condition. Regeneration in the *Bryophyllum* occurs directly from the division of the small phloem cells (pl. 18). The sections of the flax both in cross and long views show the regenerated parts arising from the epidermis cells. First the epidermis divides and then the innermost row of those cells and the stimulated cells of the region just beneath form the regenerated part, root or shoot (pl. 16).

CONCLUSIONS

Regeneration occurs in the (a) flax stem from the division of the epidermal cells; (b) *Bryophyllum* leaf at the notches from the division of the phloem cells of the veins; (c) sweet-potato, horseradish, and parsnip, from the division of the cambium cells.

FINAL CONCLUSIONS

Basing my conclusions on the instances cited in the literature and upon the laboratory experiments, I am convinced that regeneration occurs (1) from cambium cells when they are abundantly present and fully developed, as in the horseradish, sweet-potato, and parsnip, (2) from the young epidermal cells of seedlings before the central cylinder has a well-developed cambium, as in the flax seedlings, and (3) from the small and actively dividing cells of the phloem, as from the veins of the leaves of *Bryophyllum*.

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Explanation of Plate

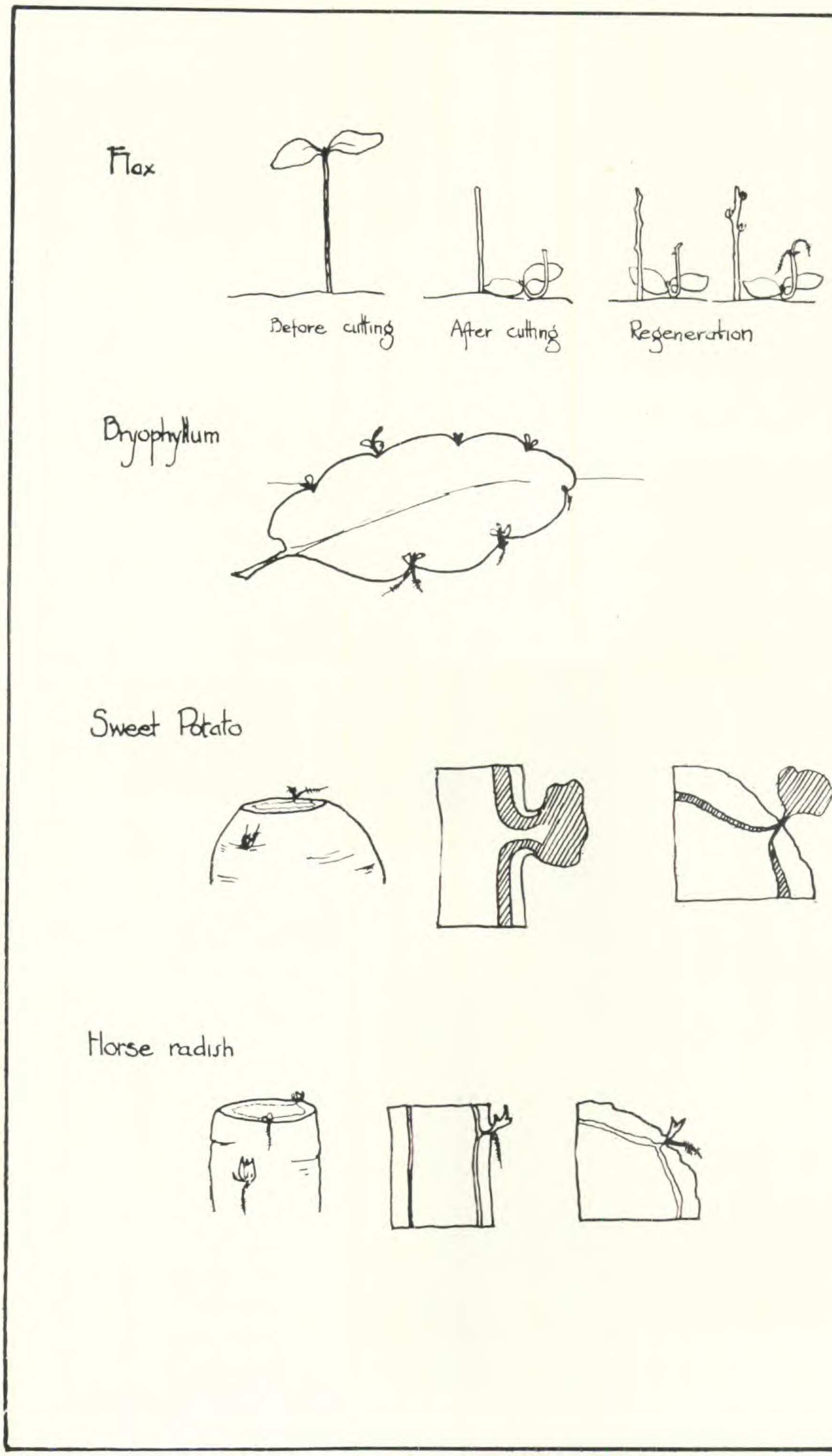
PLATE 15

The flax seedling is represented before and after cutting and shows regenerated roots and shoots.

The *Bryophyllum* leaf shows regenerated roots and shoots at the notches.

The sweet-potato has a regenerated shoot from the upper cut surface and one normally through the outer cortex.

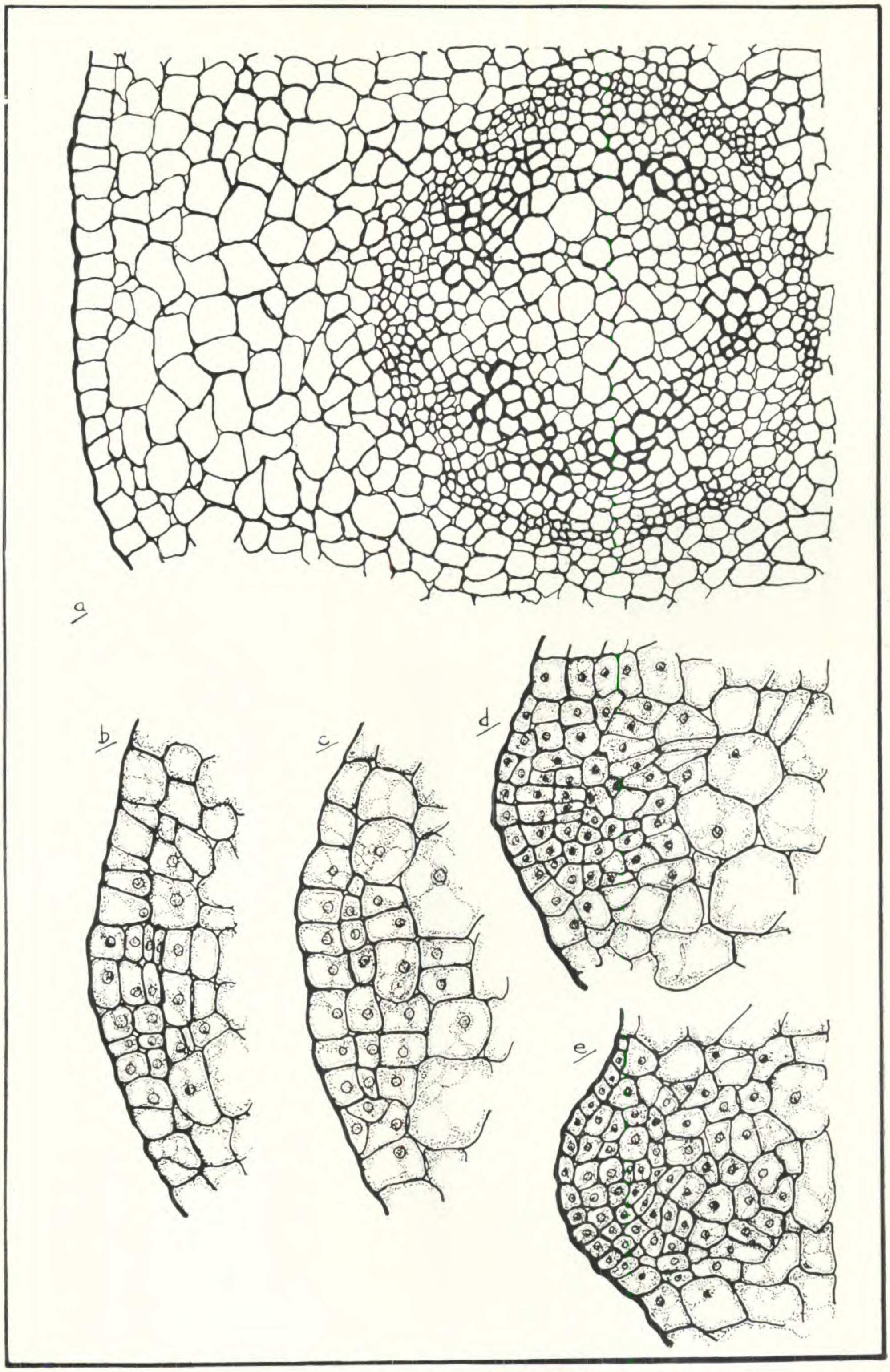
The horseradish shows its similarity to the sweet-potato.


Flax
Before cutting
After cutting
Regeneration
Bryophyllum
Sweet Potato
Horse radish

Explanation of Plate

PLATE 16

a. Normal cross-section through flax stem.
b. First divisions of epidermal cells.
c. Further divisions of inner rows formed in *b*.
d–e. Further development of regenerated bud.
(Camera-lucida drawings × 400.)

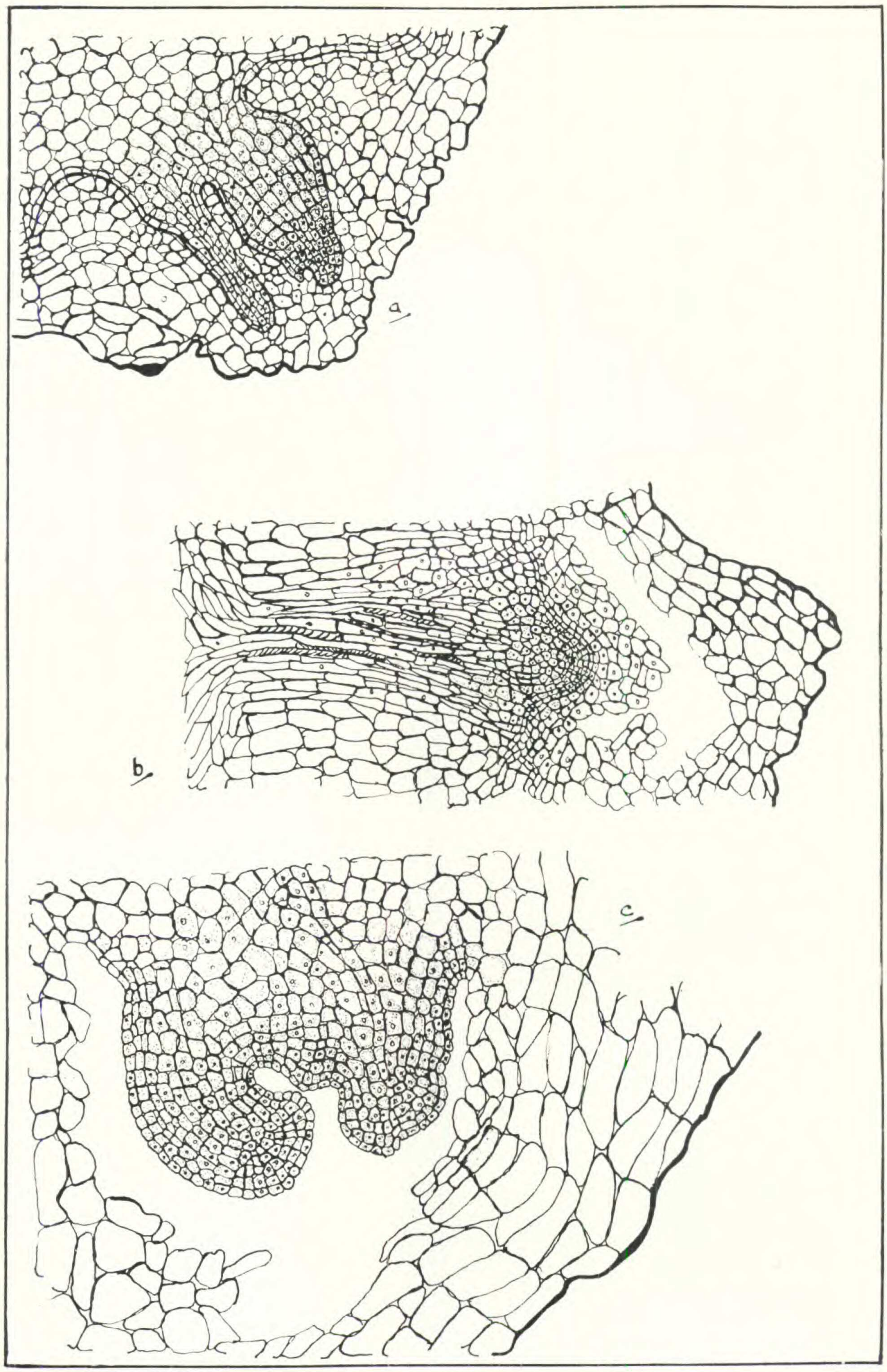


BEALS—REGENERATIVE PHENOMENA IN PLANTS

Explanation of Plate

PLATE 17

a. Regenerated root of horseradish developing from some tissue other than the epidermis or cortex.
b. Regenerated part of horseradish showing direct connection with the cambium.
c. Regenerated bud of the sweet-potato developing in a manner similar to the root in the horseradish in *a*.
(Camera-lucida outlines × 143.)



BEALS—REGENERATIVE PHENOMENA IN PLANTS

Explanation of Plate

PLATE 18

a. Cross-section of *Bryophyllum* leaf showing vein.
b. Camera-lucida drawing of vein.
c. Further division of small phloem cells of vein.
d. Regenerated root development from small cells of vein.